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MICROFABRICATED 3D SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

Microfabrication and soft lithographic techniques are combined to develop three-dimensional (3D) polydimethylsiloxane (PDMS) scaffolds comprising multiple levels of meandering pore geometry textured with 10 μm posts. Both micro-architecture and surface micro-textures have been shown to selectively stimulate cell and tissue behavior. To achieve a 3D scaffold with precise micro-architecture and surface micro-textures, 100 μm thick PDMS films were manufactured using a stacking technique to realize a 66% porous 3D structure with 200 x 400 μm horizontal through holes, 300 μm diameter vertical through holes and 71% surface coverage with 10 μm diameter and 10 μm high posts. Each PDMS porous film level was manufactured by the dual-sided molding of uncured PDMS between a three level SU-8 photoresist mold (of 200, 10, and 100 μm thick features) and a PDMS mold with 10 μm deep micro-textures. Dual-sided molding was achieved using a custom motion control mechanical jig that allowed relative mold alignment to within ~ ±10 μm.

INTRODUCTION

Traditional fabrication techniques to produce three dimensional (3D) scaffolds for tissue engineering applications such as phase separation, fiber bonding, solvent casting and particulate leaching, freeze drying, and melt molding can provide highly porous scaffolds, but have limited reproducibility and control of the micro-architecture (pore size, geometry, and distribution).[1,2] These limitations are important because specific micro- and macro-scale features within a 3D scaffold have a significant effect on multicellular structures that are required for complex tissue function.[2]

New scaffold fabrication techniques such as fused deposition modeling (FDM), selective laser sintering (SLS), 3-D printing (3-DP), and micro-stereolithography provide higher reproducibility and more precise control of the scaffold micro-architecture.[2,3] However, these techniques have limited control of the scaffold surface micro-texture, which has been demonstrated to affect a number of cell types and behaviors.[4] In addition to a controlled micro-architecture, a precise, controlled, and reproducible surface micro-texture may provide the scaffold with an even higher degree of stimulation for tissue genesis. The current study concentrates on the use of microfabrication and soft lithographic techniques to develop a 3D scaffold prototype with precise micro-architecture (architecture of the 3D structure) and surface micro-textures (surface topography) that could selectively stimulate cell and tissue regeneration. In addition, the manufacturing technique used in this study will facilitate and improve the development of 3D structures for other applications such as microfluidics, lab-on-a-chip devices, and artificial organs.

EXPERIMENTAL DETAILS

Mold Fabrication

The 3D scaffolds were produced through an innovative technique consisting of dual-sided molding and stacking of Polydimethylsiloxane (PDMS). First, a multilevel SU-8 photoresist (MicroChem Corp., Newton, MA) process was developed to produce SU-8 molds incorporating holes and posts of various dimensions. Three layers of SU-8 were processed on a standard 100 mm-diameter, 500 μm thick, n-type (100)-oriented silicon wafer as follows. First, a 200 μm thick film of SU-8 2100 was spin coated, soft baked in a *C-005* convection oven (Lindberg/Blue M, Asheville, NC) (95° C, 55 minutes), exposed (365 nm, 375 mJ/cm^2), and post exposure baked (95° C, 25 minutes). A 10 μm thick film of SU-8 2010 was then spin coated, soft baked (95° C, 5 minutes), exposed (100 mJ/cm^2), and post exposure baked (95° C, 5 minutes). Next, a 100 μm thick film of SU-8 2100 was spin coated, soft baked, exposed, and post exposure baked using the same process parameters as the first film. Finally, all three SU-8 layers were simultaneously developed in SU-8 Developer (MicroChem Corp.) (25° C, 50 minutes) using agitation, to realize a multilevel SU-8 mold with 200, 10, and 100 μm-high features (Figure 1).

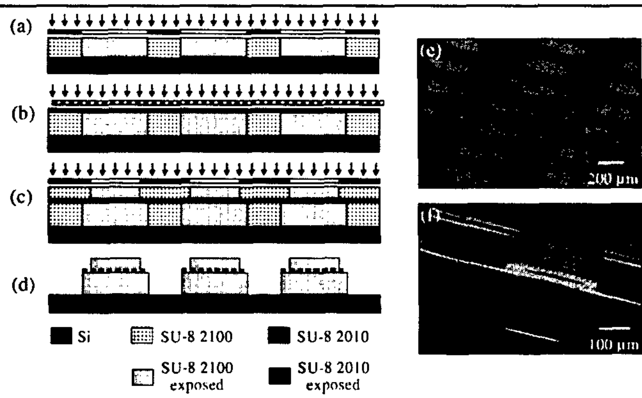

Figure 1. Processing of SU-8 molds starting with a bare silicon (Si) wafer, which is first coated with a 200 μm thick SU-8 layer and exposed to pattern the regions where the 200 μm columns will be located on the PDMS (a); followed with a coating of a 10 μm thick SU-8 layer and exposed to pattern the regions where the 10 μm posts will be located on the PDMS (b); after which, a third 100 μm thick SU-8 layer is coated and patterned where the 300 μm through holes will be located on the PDMS (c); and finally developed in SU-8 Developer to dissolve the unexposed regions (d). SEM images show a low magnification image (e) and a higher magnification (f) cross-section image of the resulting SU-8 mold.

A second mold was fabricated out of PDMS by spin coating a 10 μm thick film of SU-8 2010 on a standard 100 mm-diameter, 500 μm thick, n-type (100)-oriented silicon wafer, soft baked, exposed, and post exposure baked using the same protocol as the one used for the previous mold. The SU-8 was then developed in SU-8 Developer with agitation for 12 minutes to realize a surface with 10 μm diameter post micro-textures. Finally, this SU-8 micro-textured surface was then used to cast and realize the PDMS mold comprising 10 μm diameter holes using a method previously described.[5,6] This second mold was made of PDMS because its transparency and flexibility facilitate alignment and subsequent separation of the two molds during dual-sided molding of the final PDMS layer.

Dual-Sided Molding

The PDMS and triple-layer-patterned SU-8 molds were coated with 1H,1H,2H,2H-Perfluorodecyltrichlorosilane (Lancaster, Pelham, NH) to aid the release of the molded PDMS layer.[5,6] PDMS was mixed as previously described[5,6], poured on top of both molds, distributed to cover all the patterned areas of the molds, and degassed for 15 min. Then, both molds were placed on a custom mechanical jig (Figure 2), which allows horizontal, vertical, and rotational motion control for alignment of the molds within ~ ±10 μm.

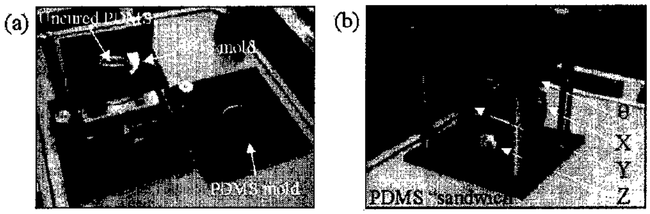

Figure 2. Motion control mechanical jig for dual-sided molding of PDMS showing the position of the SU-8 and PDMS molds with the uncured PDMS (a); and the two molds brought in contact to squeeze the PDMS (PDMS "sandwich") showing the horizontal, vertical, and rotational motion control knobs of the jig (b).

The two molds were then aligned and brought to contact while squeezing the uncured PDMS. The jig was then placed inside of an oven at 75° C for 2 hours to cure the PDMS. After curing, the two molds were removed from the jig, allowed to cool to room temperature, and immersed in methanol to remove (or separate) both molds from the squeezed PDMS (Figure 3).

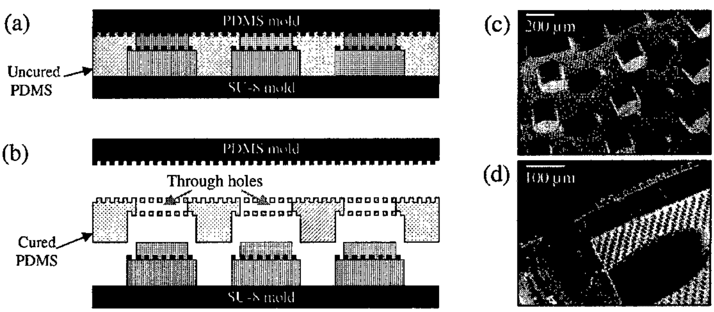

Figure 3. Dual-sided molding of PDMS with the two aligned molds in contact with each other while squeezing and molding PDMS (a); the cured PDMS layer released from the molds (b); SEM image illustrating the 300 μm diameter and 100 μm high through holes and 200 μm diameter and 200 μm high columns (c); and a closer view showing the 10 μm diameter and 10 μm high posts on both sides of the layer (d).

The resulting PDMS layer was 100 μm thick with 300 μm diameter through holes (formed at the points where both molds were in contact), 10 μm diameter posts on one side of the layer (from the PDMS mold), and 200 μm diameter and 200 μm high columns along with 10 μm diameter posts on the other side (from the triple-layer-patterned SU-8 mold). The patterned PDMS layer was then cut into ~ 1 cm x 1 cm specimens.

Stacking

The patterned PDMS layers were stacked using uncured PDMS as adhesive. PDMS was prepared as explained above, poured to cover about 2/3 of a smooth 100 mm diameter, 500 μm thick, n-type (100)-oriented silicon wafer, and spin coated using a *400 Lite* spinner (Laurell Technologies, North Wales, PA) at 4000 revolutions per minute (rpm) to achieve a ~10 μm thick layer. The cured and patterned PDMS layers were stamped on top of the uncured PDMS layer, so that the tips of the 200 μm columns were wetted with uncured PDMS. Then, the layers were handled with tweezers and stacked one on top of the other after alignment using a light microscope (Figure 4). The stacked PDMS layers were then baked at 95° C for 30 minutes to cure the PDMS (adhesive) and realize the 3D structure.

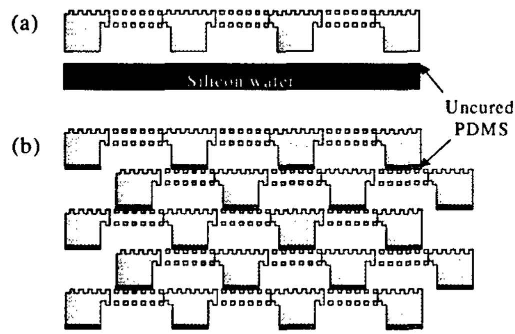

Figure 4. Stamping of PDMS layer over a 10 μm thick uncured PDMS film (adhesive) to wet the tips of the 200 μm diameter columns (a); afterwards the layer was subsequently stacked to achieve a meandering pore geometry (b). Curing of the adhesive PDMS resulted in adhesion of all the PDMS layers to realize a 3D scaffold with 66% porosity vol.% and 71% of surfaces covered with 10 μm diameter and 10 μm high posts.

RESULTS AND DISCUSSION

This paper presents a technique that combines microfabrication, soft lithography, and a custom mechanical jig to manufacture 3D structures out of PDMS, with precise micro-architecture (pore size and geometry) and surface micro-textures (surface topography) that could potentially be used as scaffolds for tissue engineering applications. PDMS is biocompatible and has been used in some implantable applications.[7] However, this technique can also be extended to create 3D structures with other tissue engineering materials such as hydroxyapatite or poly (DL-lactide-co-glycolide) (PLGA).

The fabrication process consisted of three basic steps: mold fabrication, dual-sided molding, and stacking. During mold fabrication, we developed a technique to manufacture up to four SU-8 photoresist levels using multiple exposure steps and a single developing step (Figure 5a). This approach allowed the fabrication of multiple height features of SU-8 while reducing processing time and avoiding uneven coating of previously micro-textured surfaces, which would hinder exposure uniformity, feature resolution, and alignment during the dual-sided molding of the PDMS. In addition, it allowed the manufacturing of complex geometries by using unexposed SU-8 of previously coated layers as sacrificial material for subsequent SU-8 layers (Figure 5b).

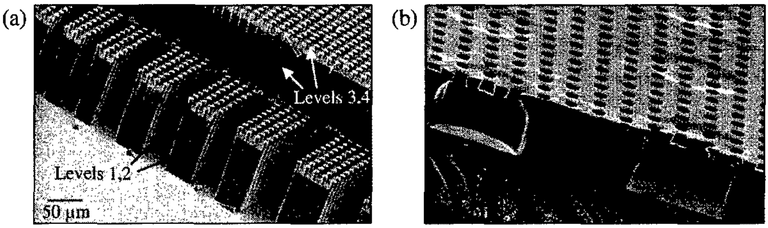

Figure 5. Four-level SU-8 structure realized with a single developing step (a); and SU-8 structure with a 30 µm thick layer comprising 40 µm diameter through holes on top of 300 µm diameter and 200 µm deep holes (b).

The multilevel SU-8 fabrication technique had a number of challenges that had to be overcome to achieve reliable manufacturing. First, as the number of SU-8 layers increased, the it was more difficult to achieve uniform SU-8 layer exposure and baking, which resulted in the lifting of SU-8 layers (Figure 6a) and underdeveloped SU-8 features (Figure 6b). Also, increased number of spin coated SU-8 layers led to the formation of thicker edge beads and higher film stresses, which resulted in wafer bowing, SU-8 cracking, uneven contact between the SU-8 and the mask, and non-uniform SU-8 exposure (Figures 6c1, c2).

Precision of the dual-sided molding was significantly enhanced by the use of a motion control mechanical jig (Figure 2). This jig allowed the alignment between the two molds within ~ ±10 µm. Although the alignment of the PDMS layers during stacking was performed manually under a light microscope, the jig could also be used when the alignment between the different PDMS layers during stacking require a higher level of resolution. During dual-sided molding, special care was given to ensuring uniform contact between the two molds. Otherwise, uneven contact due to wafer bowing and edge bead formation resulted in blocked through holes (Figure 6d) or non-uniform thickness of the PDMS layers (Figure 6e).

Because the PDMS layers are transparent, the 300 µm through holes and 200 µm high columns served as alignment marks during the stacking step with a resulting alignment within ~ ±30 µm between the stacked PDMS layers. Stamping uncured PDMS onto the 200 µm columns as adhesive is a convenient way to attach the different layers. However, care had to be taken to assure a uniform stamping/wetting of the tip of these columns, as well as subsequent contact between PDMS layers during stacking. During the stamping step, if the 200 µm diameter columns of the PDMS layers were brought into contact unevenly with the uncured PDMS (adhesive), over-wetting of some columns would result. This over-wetting lead to flowing of uncured PDMS over the columns, which, in turn, lead to obliteration of surrounding micro-textures (Figure 6f).

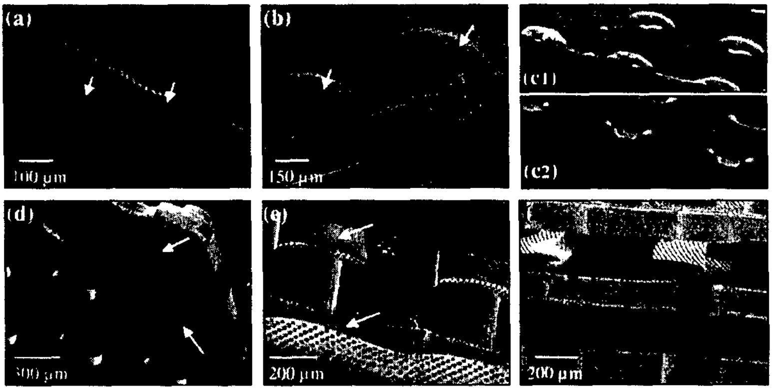

Figure 6. Challenges that were overcome with the 3D scaffold fabrication process included lifting of the SU-8 layer due to underexposure or underbaking (arrows) (a); blocking of the underlying 200 μm thick SU-8 layer by an overexposed and underdeveloped 10 μm thick SU-8 layer (arrows) (b); uneven contact between SU-8 and mask during the exposure step, which resulted in two regions of the same wafer exhibiting different kinds of underdeveloped 10 μm holes (c); uneven contact between the PDMS and SU-8 molds, which resulted in blocked 300 μm through holes (arrows) on the PDMS layer (d) and PDMS layers of different thickness (arrows) (e); and over wetting of the 200 μm columns, which led to covering of the surrounding 10 μm micro-textures (arrow) (f).

Figure 7 shows a 5 layer PDMS scaffold with 66% porosity by volume and 71% of the surfaces within the scaffold covered with 10 μm diameter and 10 μm high posts. In this particular example, the geometry and size of these 10 μm posts were chosen because they have been shown to enhance mesenchymal stem cell growth in culture.[5,6] However, micro-textures of different geometries can be specifically designed to selectively direct and stimulate other kinds of cells and tissues within precise locations of the 3D scaffold.

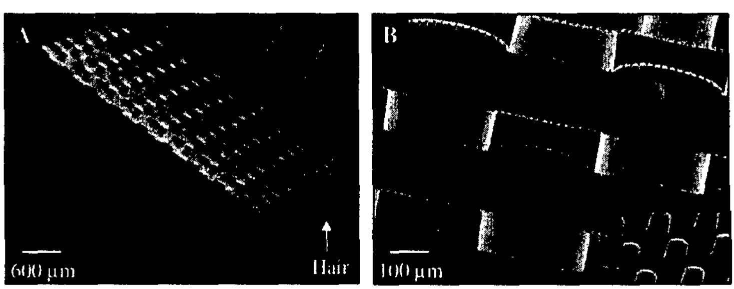

Figure 7. A five-layer PDMS scaffold on a penny (a); and a closer view of the cross-section showing the alignment between adjacent layers that resulted in a meandering pore geometry (b). Insert depicts the 10 μm diameter and 10 μm high posts present on all horizontal surfaces (71% of all surfaces within the 3D scaffold).

CONCLUSIONS

This study describes the processing and limitations of manufacturing 3D scaffolds with both precise micro-architecture and surface micro-textures for tissue engineering applications. These precise geometrical features can stimulate specific biological responses at both the cell and tissue levels, increasing the tissue genesis potential of the 3D scaffold. The fabrication process reported here combines microfabrication and soft lithography into an innovative dual-sided molding and stacking technique of PDMS layers. This technique consists of three major steps including a) mold fabrication using multilevel SU-8 photoresist processing; b) dual sided molding of PDMS using a custom mechanical jig for precise motion control of the molds; and c) alignment and stacking of PDMS layers. This process allows the fabrication of 3D structures with features of multiple dimensions and micrometer resolution.

ACKNOWLEDGMENTS

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REFERENCES

1. Gabriel-Chu TM, Orton DG, Hollister SJ, Feinberg SE, Halloran JW. Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures. Biomaterials 2002; 23:1283-1293.
2. Tsang VL, Bhatia SN. Three-dimensional tissue engineering. Advanced Drug Delivery Reviews 2004; 56:1635-1647.
3. Salgado AJ, Coutinho OP, Reis RL. Bone tissue engineering: State of the art and future trends. Macromolecular Biosciences 2004; 4:743-765.
4. Curtis A, Wilkinson C. Topographical control of cells. Biomaterials 1998; 18:1573-1583.
5. Mata A, Boehm C, Fleischman A, Muschler GF, Roy S. Analysis of connective tissue progenitor cell behavior on various formulations of polydimethylsiloxane smooth and channel micro-textures. Biomedical Microdevices 2002; 4; 4:267-275.
6. Mata A, Boehm C, Fleischman A, Muschler GF, Roy S. Growth of connective tissue progenitor cells on micro-textured polydimethylsiloxane surfaces. Journal of Biomedical Materials Research 2002; 62:499-506.
7. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials Science: An Introduction to Materials in Medicine. Academic Press, San Diego, California, 1996; p.59.